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Mutant Metabolic Enzymes Are at the Origin of Gliomas

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Abstract

Mutations of the *isocitrate dehydrogenase (IDH)* metabolic enzymes *IDH1* and *IDH2* have been found to be frequent and early genetic alterations in astrocytomas and oligodendrogliomas. All mutations identified to date affect a single amino acid located within the isocitrate binding site (R132 of *IDH1* and the analogous R172 residue of *IDH2*). *IDH1* and *IDH2* mutations define a specific subtype of gliomas and may have significant utility for the diagnosis, prognosis, and treatment of patients with these tumors.

Identification of *IDH1* as a novel GBM gene

Malignant gliomas are the most frequent and lethal tumors of the central nervous system, and glioblastoma multiforme (GBM; WHO Grade IV astrocytoma) is the most biologically aggressive subtype. GBMs may arise de novo (“primary GBM”) or develop in the setting of a lower-grade glioma (“secondary GBM”) (1). An unbiased, genome-wide analysis of the somatic mutations occurring in GBMs revealed recurrent mutations in R132, the active site of *IDH1*, a gene with no known link to gliomas, in 12% of tumors analyzed (2). Intriguingly, mutations of *IDH1* predominantly occurred in younger patients, were associated with a better prognosis, and were preferentially found in tumors that possessed *TP53* mutations but lacked other common GBM alterations: all characteristics of secondary GBMs. Additional studies have confirmed that *IDH1* is mutated in >80% of secondary GBMs (3–8), whereas less than 10% of primary GBMs harbor these alterations.

Mutant *IDH1* and *IDH2* as astrocytoma- and oligodendroglioma-specific gatekeepers

To evaluate the timing of *IDH1* alterations in glioma development, *IDH1* mutations have been assessed in a large number of gliomas of various types, and the results (confirmed by multiple investigators) are striking: mutation of *IDH1* occurs early in glioma progression, with somatic mutations of the R132 residue of *IDH1* identified in the majority (>70%) of Grades II and III astrocytomas and oligodendrogliomas, as well as in secondary GBMs that develop from these lower grade lesions (2–10). In addition, mutation analysis of the closely related *IDH2* has revealed recurrent somatic mutations of *IDH2* residue R172, with most mutations occurring in tumors lacking *IDH1* mutations (5,9). The R172 residue in *IDH2* is the exact analogue of the frequently mutated R132 residue of *IDH1*: Both are conserved in all known species and form hydrogen bonds with the isocitrate substrate (11).

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Astrocytomas and oligodendrogliomas both contain frequent *IDH1* or *IDH2* mutations but do not share other genetic alterations that occur early in the development of these two glioma lineages. For example, the majority of low-grade diffuse astrocytomas contain both an *IDH* mutation and a *TP53* mutation, whereas most oligodendrogliomas have both *IDH* mutations and 1p/19q loss (3,5,6,8). One study revealed that, of 23 grade II astrocytomas with both *IDH* and *TP53* mutations examined, 17 samples have both *IDH* and *TP53* mutations, three have *IDH* mutations but do not contain *TP53* mutations, and three have neither *IDH* nor *TP53* mutations (5). In addition, Watanabe et al. dissected multiple biopsies from the same patients and found that *IDH1* mutations always preceded the acquisition of a *TP53* mutation or loss of 1p/19q (8). This genetic evidence suggests that *IDH* mutations are early genetic events in the development of a glioma from a cell-of-origin that can give rise to both astrocytes and oligodendrocytes. That *IDH* mutations were not identified in any WHO Grade I pilocytic astrocytomas (3,5,12), which rarely undergo malignant transformation, indicates that these tumors arise through a different mechanism.

IDH1 and *IDH2* mutations are remarkably specific to Grades II and III astrocytomas, oligodendrogliomas, and secondary GBMs. They are not found in ependymomas and are found only rarely in pilocytic (Grade I) astrocytomas (3,5,12). Most recently, *IDH1* R132 mutation have been found in 15 of 187 acute myeloid leukemia samples (AML) (13). The only tumors other than astrocytomas, oligodendrogliomas and AMLs in which *IDH1* mutations have been reported are a single case of colorectal cancer (14), two prostate carcinomas (7), and a minority of analyzed cases of adult supratentorial primitive neuroectodermal tumors (3,10).

Gliomas with *IDH* mutations are a different subtype of disease

The pattern of other genetic mutations found in gliomas with *IDH* mutations is entirely different than that in gliomas with wild-type *IDH1* and *IDH2* (Figure 1). Nearly all of the anaplastic astrocytomas and GBMs with mutated *IDH* genes were also found to have mutation of *TP53* (80%), but only 5% had alterations in any of the common GBM genes *PTEN*, *EGFR*, or *CDKN2A/CDKN2B*. Conversely, anaplastic astrocytomas and GBMs with wild-type *IDH1* and *IDH2* had relatively few *TP53* mutations (20%) and extremely frequent alterations of *PTEN*, *EGFR*, or *CDKN2A/CDKN2B* (74%). Similarly, loss of 1p/19q was observed in 85% of the oligodendrocytic tumors with mutated *IDH* but in none of the patients with wild-type *IDH1* and *IDH2* (3,5,6,8).

Clinically, glioma patients with *IDH* mutations are also distinct from those with wild-type *IDH1* and *IDH2*. For example, adult patients with anaplastic astrocytomas and GBMs with *IDH* mutations are significantly younger than those with wild-type *IDH1* and *IDH2* (median age of 34 vs. 56 for anaplastic astrocytomas and 32 vs. 59 for GBMs). However, no *IDH* mutations have been identified in pediatric glioblastomas, and children with *IDH*-mutated low-grade gliomas are older than the others as well (median age 17y vs. 5y) (5,6,15). GBM patients with *IDH* mutations have a median overall survival of 31 months, significantly longer than the 15-month survival in patients with wild-type *IDH1* and *IDH2* (5). Although both younger age and mutated *TP53* are positive prognostic factors for GBM patients, this association between *IDH1* mutation and improved survival is noted even in the subgroup of young patients with *TP53* mutations (2). Mutations of *IDH* are also associated with improved prognosis in patients with anaplastic astrocytomas, whose median overall survival is 65 months for patients with mutations and 20 months for those without (5). A multivariate analysis has confirmed that *IDH1* mutation was an independent favorable prognostic marker after adjustment for grade, age, MGMT status, genomic profile, and treatment (16).

Functional characterization of *IDH* mutations

The recurrence of mutations at a specific site on each gene in a heterozygous fashion is reminiscent of activating alterations in oncogenes such as *BRAF*, *KRAS*, and *PIK3CA*. However, biochemical studies have provided evidence that mutant IDH may act in a dominant-negative fashion to inhibit catalytic activity of the enzymes. The IDH1 and IDH2 enzymes catalyze the oxidative decarboxylation of isocitrate to α -ketoglutarate, generating NADPH from NADP⁺. IDH1 is localized in the cytoplasm and peroxisomes, whereas IDH2 is localized in the mitochondria and participates in the citric acid cycle for energy production. Modeling studies based upon the human cytosolic IDH1 crystal structure suggest that substitution of R132 with any one of the six amino acids observed thus far in gliomas would impair interactions of the enzyme with isocitrate (17). Biochemical analyses of recombinant IDH1 proteins revealed that mutant IDH1 had a dramatically reduced affinity for isocitrate (17). In addition, by assaying the reduction of NADP⁺ to NADPH, all *IDH1* and *IDH2* mutations identified in patients were found to abrogate their enzymatic activity (5,17). Furthermore, the wild-type IDH1 and mutant (R132H) IDH1 was reported to form a heterodimer, which exhibited only 4% of the activity of the wild-type enzyme when assayed with limited isocitrate concentration and resulted in decreased α -ketoglutarate levels (17).

Metabolic enzymes as cancer genes and the Warburg effect

The biochemical studies of IDH1 have also shed some light on the possible underlying mechanism by which mutations in metabolic pathways contribute to the pathogenesis of gliomas. Hypoxia-inducible factor (HIF) is a master regulator of genes that are activated by low oxygen levels and regulates the expression of genes implicated in glucose metabolism, angiogenesis, and other signaling pathways critical to tumor growth. Prolylhydroxylases hydroxylate and promote the degradation of HIF-1 in the presence of oxygen using α -ketoglutarate and iron as cofactors (18). Further, reduction in IDH1 activity produces a reduction in α -ketoglutarate levels that in turn can lead to stabilization of HIF-1 α , activation of the HIF pathway, and contribution to gliomagenesis (17). Nevertheless, this connection to HIF-1 may only partially account for the pathological function of *IDH* mutations in gliomas. Although the *in vitro* biochemical results demonstrate an effect of the mutations on *IDH* function, they do not necessarily mean that the mutations are inactivating: It is possible that the mutations have a different pathogenic effect, such as alteration of affinity for a substrate other than isocitrate. In addition to reducing α -ketoglutarate levels, IDH1 and IDH2 contribute to NADPH production in cells. NADPH is required for the synthesis of glutathione, which protects cells from redox stress. There is still a paucity of biological data to support that decreased production of NADPH caused by *IDH* mutations may confer a survival advantage.

In addition to *IDH* mutations in gliomas, mutations in metabolic pathways occur in other cancers. Fumarate hydratase and succinate dehydrogenase have been shown to act as tumor suppressors of paraganglioma and leiomyoma, respectively (19). Accumulation of multiple genetic alterations in cancer cells results in dysregulation of various cellular pathways, some of which may modulate cellular metabolism (20). Otto Warburg observed that in most cancer cells, energy is produced predominantly by aerobic glycolysis in the cytosol, rather than by oxidation of pyruvate in mitochondria, as in most normal cells. He postulated that this change in metabolism is the fundamental cause of cancer (21). It has been proposed that aerobic glycolysis provides cancer cells with growth advantage by supplying needed metabolites for incorporation into the biomass to produce a new cell (22). One mechanism through which *IDH* mutations contribute to the Warburg phenomenon in glioma cells may be through changing metabolome or metabolite pools and thereby facilitating glycolytic flux. During glioma progression, the glycolytic traits found in these invasive cancer cells may arise as

adaptive mechanisms to environmental constraints in which specific nutrients or oxygen may be limiting.

Conclusions

IDH mutations appear to play a central role in the pathogenesis of gliomas and define a specific subtype of glial tumors. This knowledge indicates opportunities to improve diagnostic and therapeutic strategies for gliomas, which are not currently targeted at the specific molecular alterations present in a particular tumor. The localization of *IDH1* and *IDH2* mutations to a single amino acid (R132 and R172, respectively) should simplify the use of this genetic alteration to distinguish *IDH1* or *IDH2* -mutated and *IDH1* and *IDH2*-wild-type gliomas and guide clinical treatment. Furthermore, metabolic profiling of glioma cells with *IDH* mutations could reveal new clues about the cancer-specific bioenergetics of these tumors and about novel strategies for therapeutic intervention.

However, any such improvements in the treatment for patients with *IDH*-mutated gliomas will hinge on a better understanding of the functional role of the mutant *IDH* in the pathogenesis of these tumors. Further analysis of *IDH1* and *IDH2* in glioma model systems will be necessary to clarify the genetic mechanisms involved in the initiation and malignant progression of this disease. These studies are anticipated to lead to an improved molecular classification of gliomas and should help establish these mutant genes and the related metabolic pathways as attractive targets for therapeutic intervention.

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Figure 1. Model of malignant glioma development

For each tumor type, common genetic alterations (*IDH1* or *IDH2* mutation, *TP53* mutation, 1p 19q loss, and *CDKN2A* loss) are indicated. Detailed frequencies of genetic alterations are provided in Supplementary Table 1 of the literature (5). In general, tumors to the right of the red vertical line harbor *IDH1* or *IDH2* alterations while those on the left do not.